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Comparison of Aquaponics and Hydroponics on Basil (*Ocimum basilicum*) Morphometrics and Essential Oil Composition

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Cover Page Footnote
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I. Introduction

World population growth is increasing rapidly at a rate which the amount of agricultural land available for food production cannot match, resulting in an increasing need for alternative methods of food production (de Carvalho et al. 2015). While various methods of soilless farming have been used from the time of early civilizations, the hydroponic method of farming has been in modern food production for just decades, and the modern closed-loop aquaponic system was first described in 1984 (Elia et al. 2014). Both types of systems allow for a wide variety of vegetables and herbs to be grown (the most popular herbs are shown in Table 1) and are adaptable to numerous diets and cultures.

Table 1: Common herbs grown in hydroponic and aquaponic systems.

Plant		Comments	References
Basil (*Ocimum basilicum*)		Low to medium nutritional requirements; well adapted to aquaponic systems Often grown using aquaponics Recommended because of its quick growth, adaptability, and various uses pH: 5.5-6.5 Temp Range: 18-30°C Optimal Temp: 20-25°C Spacing: 15-25 cm Light Exposure: sunny or slightly sheltered Growth Time: 5-6 weeks	Diver, 2006 Elia et al., 2014 Moya et al., 2014 Somerville et al., 2014
Mint (*Mentha spp.*)	Peppermint (*M. piperita)* Spearmint (*M. spicata)*	Often grown using aquaponics Recommended because of their quick growth, adaptability, and various uses	Elia et al., 2014 Moya et al., 2014
Chives (*Allium schoenoprasum*)		Low to medium nutritional requirements; well adapted to aquaponic systems Often grown using aquaponics	Diver, 2006 Elia et al., 2014

Hydroponic farming is an agricultural method used since ancient times (Schafer 2014) that utilizes nutrient-rich water instead of soil to grow plants (Giurgiu et al. 2014; Schafer 2014). In hydroponics, water is supplemented with macro- and micronutrients necessary for plant growth such as nitrogen, calcium, potassium, sodium, magnesium, and iron (de Carvalho et al. 2015; Lazar et al. 2015). Methods vary in ways nutrients are delivered to plants; in general, seeds are planted in a substrate that is inorganic (such as river stone, rock wool, perlite, vermiculite, gravel, or clay pebbles), or organic (such as coconut fibers, peat moss, or cocopeat) (Roosta and Afsharipoor 2012; Giurgiu et al. 2014; Moya et al. 2014; Schafer 2014; Lazar et al. 2015). In some systems, plants are removed from the substrate once roots emerge, which are suspended directly into the aqueous solutions, while in others the seedlings may be kept in the substrate with various methods of delivering nutrients (Schafer 2014). These methods include systematically spraying or washing the roots with aqueous solution, or irrigating the solution through porous substrate (Roosta and Afsharipoor 2012; Schafer 2014).

The concept of aquaponics, using fish waste to fertilize plants, has been utilized for thousands of years with applications in early Asian and South American civilizations (Somerville et al. 2014). However, it was not until the 1970s and 1980s that academic research of this idea was incorporated into contemporary food production systems (Watten and Busch 1984). In aquaponics, fish waste products provide nutrients needed by plants, which act as bio-filters and maintain a clean environment for the fish (Elia et al. 2014; Moya et al. 2014; Tomlinson 2015).

One of the most critical macronutrients required for vegetative growth is nitrogen, found in fish waste in the form of ammonia (NH_3). Though NH_3 is toxic to fish in high concentrations, it can be oxidized into nitrite (NO_2^-) by nitrifying bacteria (*Nitrosonomas*) and subsequently oxidized again by a second type of nitrifying bacteria (*Nitrobacter*) into nitrate (NO_3) that is easily absorbed by plants (Tomlinson 2015; Somerville et al. 2014; see Figure 1). The entire process occurs accordingly: (A) $NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$, (B) $NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^+$. Thus, aquaponics utilizes a closed-loop system: water is re-circulated from plants to fish and waste from one component of the system becomes a resource for another part, and vice-versa (Moya et al. 2014; Tomlinson 2015).

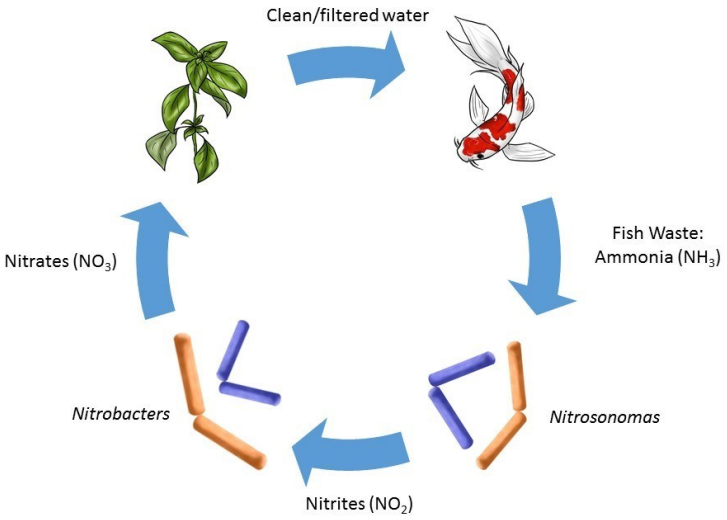

Figure 1: Nitrogen cycling in aquaponic systems

While any fish species may be used as a source of ammonia, many commercial fish species thrive in both backyard and commercial aquaponics (Table 2). Once introduced into the aquaponic system, fish require a source of food and routine monitoring for illness. Because the aquaponic system can be a source of both animal and plant protein, the potential for food and economic yield may be higher. However, studies evaluating aquaponics compared to hydroponics are lacking (Love et al. 2014).

The aromatic nature of basil is due to the composition of essential oils. These essential oils are elicited as a stress response and serve as antimicrobial (Lahariya and Rao 1979) and antifungal (Dube et al. 1989) agents. Some of the highest concentration essential oils in basil include eugenol, camphor, linalool, thymol, and methyl chavicol (Kruger et al. 2002). As a result, basil has been used medicinally to treat coughs, diarrhea, and kidney malfunctions (Simon et al. 1999).

The objectives of this project are to compare: (1) water nutrient profiles in aquaponic and hydroponic systems over time, (2) basil (*Ocimum basilicum* var. *Eleonora*) harvest yields in both aquaponic and hydroponic systems to determine if the added complexity of an aquaponic system is justified and (3) essential oil composition of basil in both systems. Because it is expected that aquaponic nutrient profiles will be less variable compared to hydroponic water nutrient profiles, it is hypothesized that basil yield will be higher in the aquaponics system and that essential oil composition will differ.

Table 2: Common fish species raised in aquaponic systems

Fish	Ideal Temp	Time to Maturity	References
Tilapia (*Oreochromis spp.*)	27-30°C	6-8 months	Diver, 2006 Elia et al., 2014 Somerville et al., 2014
Carp (family Cyprinidae)[1]	25-30°C	9-11 months	Elia et al., 2014 Somerville et al., 2014
Catfish (order Siluriformes)	26°C	9-10 months	Elia et al., 2014 Somerville et al., 2014
Rainbow trout (*Oncorhynchus mykiss*)	10-18°C	14-16 months	Diver, 2006 Elia et al., 2014 Somerville et al., 2014
Giant river prawn (*Macrobrachium rosenbergii*)	24-31°C	4 months	Somerville et al., 2014

[1] Cyprinids include ornamental fish such as goldfish (*Carassius auratus*) and koi (*Cyprinus carpio haematopterus*) that are often used in aquaponics.

II. Materials and Methods

This study was conducted at a Special Growers greenhouse located in Maryville, Tennessee. The facility contained functioning hydroponic systems at the start of the study and was the site at which an aquaponic system was built. The established hydroponic systems were comprised of a series of lateral grow beds connected to a water basin situated beneath them. Nutrient-supplemented water was pumped from the water basin to the higher end of the grow bed, where it was then pulled through the bed (and so, through the root systems) to the lower end and drained back into the basin. Nutrients in the water were kept

at 1000 ±100 ppm parts per million (ppm), with equal parts of FloraMicro, FloraGro, and FloraBloom (General Hydroponics, Santa Rosa, CA). The pH level was kept at 6.0 ± 0.2 using pH Up and pH Down (General Hydroponics, Santa Rosa, CA).

Establishment of Aquaponics System

The aquaponic system was constructed following Malcolm and Arcaro (2011; pgs. 21-25) with Sunleaves Rocks (Sunleaves Garden Products, Bloomington, IN) used as media for the grow bed. Once the water was dechlorinated, 6.25 g of ammonia and 125 ml of Microbe-Lift Nite-Out II bacteria (*Nitrosomonas, Nitrospira, and Nitrobacter*; Microbe-Lift, Malverne, NY) were added to the system on Day 1 of cycling. System levels (pH, temperature, ammonia, nitrite, nitrate) were recorded daily using API Freshwater Master Test Kit (Mars Fishcare, Chalfont, PA) and until the system became "established" (i.e. there were measurable amounts of nitrates as well as close to no ammonia or nitrites). pH was maintained at 7.0 ± 0.2 (up to 7.8 if no plants) using pH Up and pH Down (General Hydroponics, Santa Rosa, CA), and bacteria were added on days 1, 3, and 16. When the aquaponics system had measurable levels of nitrates and levels of ammonia and nitrites were zero, six koi ranging from 20-50 cm in size were added to the system. Koi were obtained from Perennial Ponds in Maryville, Tn and allowed to acclimate for 3 hours before basil plants were introduced to the grow beds.

Experimental Design

To initiate the experiment, basil plants (*Ocimum basilicum* var. *Eleonora*) were divided into four treatments (n = 12 for each treatment) based on system type (hydroponics or aquaponics, determined by random selection) and age when added to its respective system: 4-week old "young" plants or 6-week old "old" plants. Plants in the aquaponic grow bed were arranged in a 6 x 4 grid spaced 20.32 cm apart in both x and y planes, whereas those in the hydroponic grow beds were placed single file 20.32 cm apart, with 20.32 cm in between each grow bed in order to have equal growing space in each system (see Figure 2A-B). Plants were numbered as they were entered into their respective systems for identification. Plants #1-12 were young plants, and plants #13-24 were old plants. Each basil plant was measured for stem length and number of leaves after being planted in the

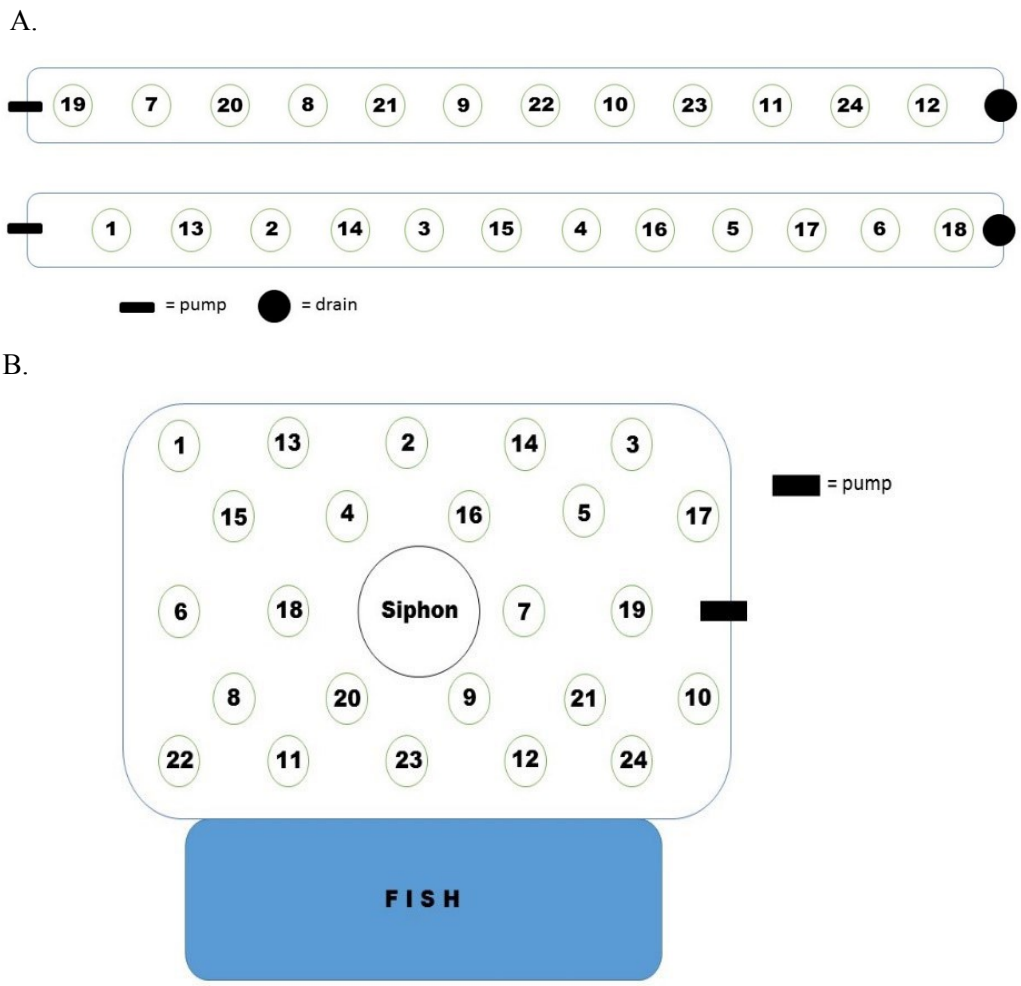

Figure 2: (A) Hydroponic and (B) aquaponic system designs. Numbers indicate the ID of the plant.

grow bed. Stem length was measured as distance between the shoot apical meristem and the top of the media bed.

The aquaponic and hydroponic systems were arranged juxtapose to each other in the same greenhouse to minimize external confounding influences. pH was maintained higher at 6.8 – 7.2 in the aquaponic system rather than at the 5.8 – 6.2 range of the hydroponic system as the former must appease not only plants but also fish and bacteria.

Plants were allowed to grow for 4 weeks. For water analyses, 4 ml samples from each system were taken over the growing period (Days 0, 11, 17, 24, 28). Samples were analyzed

with ion chromatography using a Metrohm Dual Channel 850 IC (Metrohm, Herisau, Switzerland) by Cornerstone Analytical Laboratories (Maryville, TN). Anions were separated on a MetroSep A Sup 5 (150mm length/4.mm ID) column. The eluent contained 3.2 mM carbonate and 1.0 mM bicarbonate with a flow rate of 0.7ml/min. The analytes were detected using a chemically suppressed conductivity dectector using a 100mM sulfuric acid suppressant. Cations were separated on a MetroSep C4 (150mm length/4.0mm ID) column. The elutent contained 1.7mM nitric acid and 0.7mM dipicolinic acid with a flow rate of 0.9ml/min. The analytes were detected using a conductivity detector. The detector response was calibrated using IC standards for cations (Thermo # 040187 and Sigma #101684894) and anions (Thermo #056933 and 057590), diluted to give a calibration curve in the range between 0.5-50ppm.

Each plant was measured for stem length and number of leaves after 2 and 4-weeks of growth. After measurements were taken at the 4-week interval, 2 leaves were taken from each "old" plant for essential oil analysis.

Essential oil analysis was conducted by gas chromatography-mass spectrometry (GCMS). Briefly, collected leaves were minced and extracted with anhydrous diethyl ether for three days. Leaves were removed by a filter. The extract was dried using anhydrous magnesium sulfate and evaporated under reduced pressure using a rotary evaporator. The total extract weight was recorded and diluted to 10 mg/ml. One ml extracts were analyzed by GCMS by Cornerstone Analytical Laboratories (Maryville, TN) using an Agilent 6890 GC with a 5972 MS (temperature program 40→300 ºC @ 5 ºC/min, held at 300ºC for 8 minutes with a 60 min run at 2 microliter injection volume). Compounds were tentatively assigned using the NIST08 MS library.

Statistical Analysis

Leaf density was calculated as (number of leaves/stem height). A t-test assuming equal variance (with $\alpha = 0.05$) was performed for each measurement (number of leaves, stem height, and leaf density) to determine meaningful differences between each treatment.

III. Results

Water Quality Analyses

Analyses of the water quality in each system revealed differences in the ion composition of water between systems (Figure 3). Concentrations of all ions differed in each system, with ions in the aquaponics being lower than those in hydroponics.

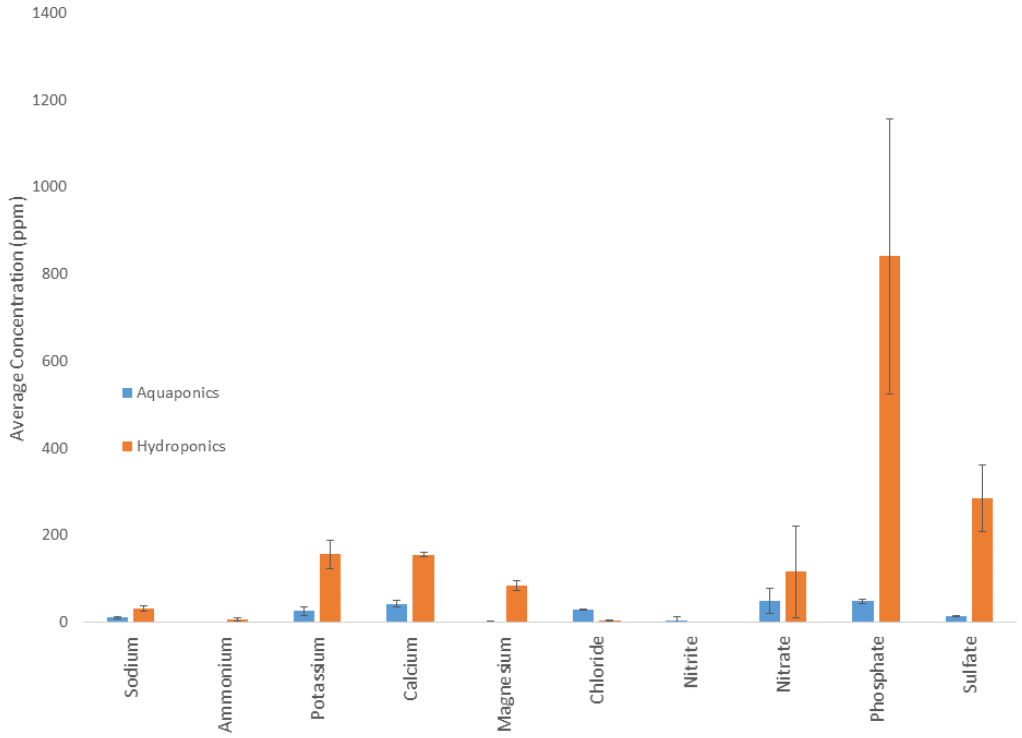

Figure 3: Comparison of the average ion concentrations (+/- SD) for each species measured over the study period.

Additionally, both systems showed changes over time (Figure 4). In the hydroponic system, cation concentrations changed minimally over time, but anions had large increases in nitrate, phosphate and sulfate over the study period. The aquaponics system showed some changes in the concentrations of specific cations over the study period. Ammonium concentration dropped close to zero, while potassium and calcium levels increased. Aquaponics anions also showed increasing changes over time. For instance, the nitrate level steadily climbed over time whereas the nitrite level decreased to negligible levels.

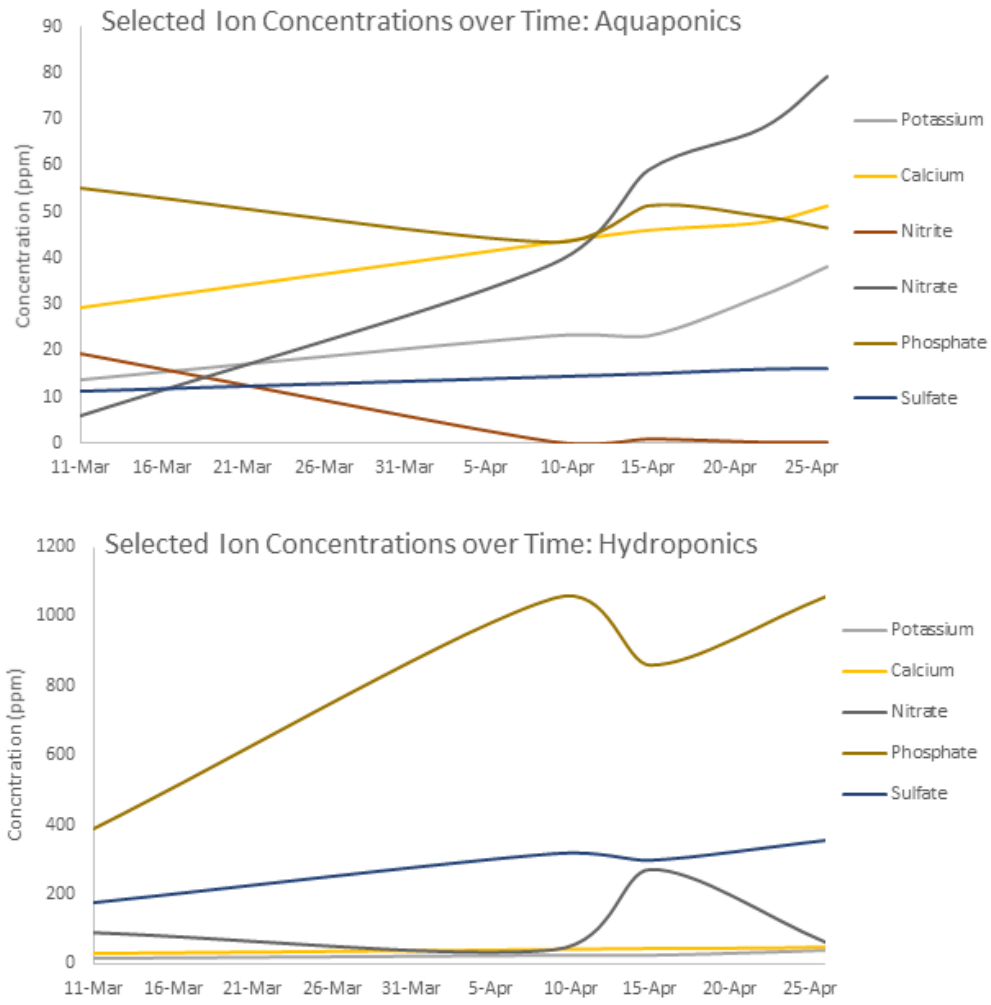

Figure 4: Fluctuations of selected ions in both aquaponic and hydroponic systems.

Plant Morphometrics

Of the twelve comparisons made between measurements of plants in aquaponic and hydroponic systems, four were found to be significant (see Table 3). Significant values included leaf density in old plants measured at 2 weeks, number of leaves in old plants measured at 2 weeks, number of leaves in young plants measured at 4 weeks, and stem length in young plants measured at 4 weeks. Of these variations in mean measurements, two of the four favored aquaponic systems (number of leaves and leaf density of old plants measured at 2 weeks) and the other two favored hydroponic systems (number of leaves and stem length in young plants 4 weeks). By two weeks, basil plants in the aquaponics system had more aphids and aphid damage compared to the hydroponics system (an example is shown in Figure 5). All other visible parameters, including leaf color, appeared the same.

Table 3: Measurements (mean ± standard error) of leaf number, stem height, and leaf density (leaf number/stem height) for basil plants grown in either aquaponic (n = 12 for each age group) or hydroponic (n = 12 for each age group) systems. "Young" plants were placed in their respective system at 4 weeks old and "old" plants at 6 weeks old. Measurements were taken after 2 weeks and 4 weeks of growth in the system. Bolded values are significant.

Leaf Number				
		Aquaponics	Hydroponics	*p*-value
Young	2 weeks	16.17 ± 1.04	17.00 ± 0.69	0.5093
Young	4 weeks	28.67 ± 1.79	40.67 ± 2.72	**0.0013**
Old	2 weeks	32.75 ± 2.44	20.75 ± 1.03	**0.0002**
Old	4 weeks	52.58 ± 3.47	54.42 ± 2.35	0.6661
Stem Height (cm)				
		Aquaponics	Hydroponics	*p*-value
Young	2 weeks	31.88 ± 2.16	34.68 ± 3.46	0.4987
Young	4 weeks	59.8 ± 4.09	85.97 ± 7.08	**0.0041**
Old	2 weeks	58.96 ± 4.88	54.1 ± 3.77	0.4396
Old	4 weeks	103.7833 ± 8.62	120.82 ± 11.90	0.2588
Leaf Density				
		Aquaponics	Hydroponics	*p*-value
Young	2 weeks	0.53 ± 0.04	0.54 ± 0.05	0.9103
Young	4 weeks	0.49 ± 0.02	0.49 ± 0.04	0.8896
Old	2 weeks	0.58 ± 0.05	0.40 ± 0.03	**0.0036**
Old	4 weeks	0.52 ± 0.03	0.49 ± 0.04	0.5186

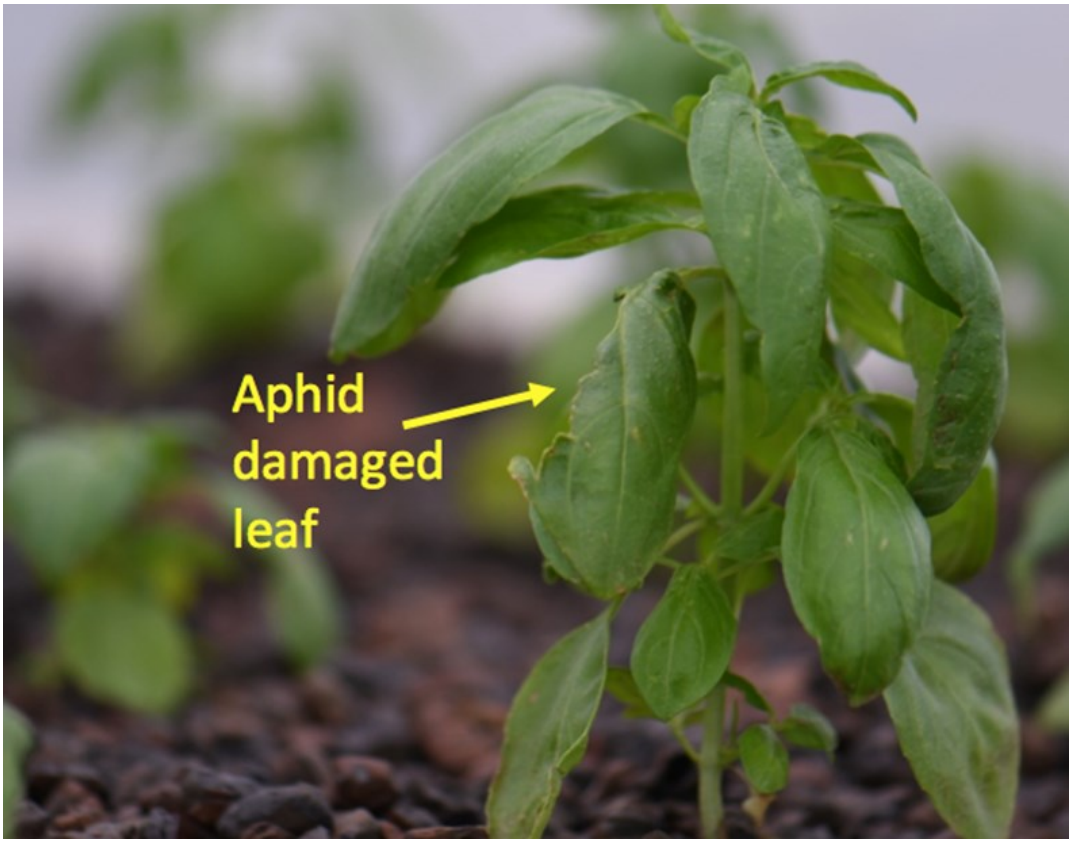

Figure 5: Aphid damage in an aquaponics basil plant.

Essential Oil Analysis

Through comparison of the GC-MS data from the leaf extracts from each system with the NIST08 GC-MS library, multiple compounds were tentatively identified and were found to be present in the leaves from both systems. There were some differences in the apparent concentrations of the individual compounds in each extract. The essential oil composition analysis of leaves from each system indicated leaves from plants grown in the hydroponic system had higher concentrations of essential oils eucalyptol, linalool, eugenol, and cadinols (see Figure 6). The largest differences were seen in the concentrations of eugenol and the cadinols.

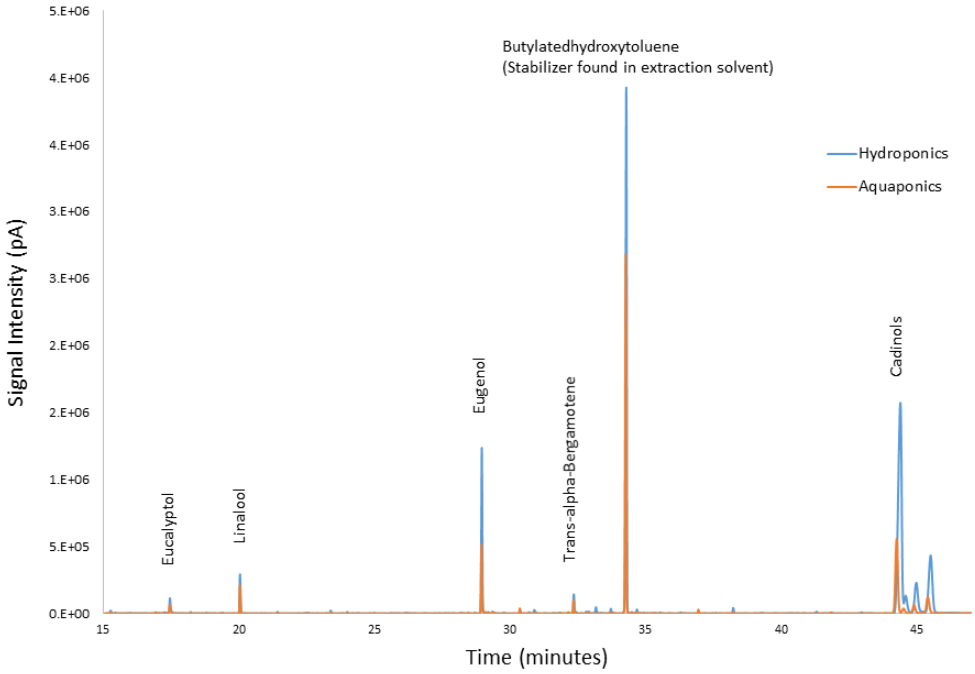

Figure 6: Chromatogram of volatile basil extract from both aquaponics and hydroponics.

IV. Discussion

While there are anecdotal reports of aquaponics techniques being superior to hydroponic methods in vegetable production (e.g., Wilson 2005), controlled studies are lacking. Here, we show that (1) aquaponics provided a more constant supply of nutrients to plants when compared to hydroponics, (2) there were significant differences in leaf number and plant height, but these differences depended on age of the plants when introduced into the system, and (3) the essential oil composition is different in basil grown in hydroponic and aquaponic systems.

Results from water chemistry analysis indicated that the aquaponics system was functioning as expected. The pH of the aquaponic system was maintained at a slightly higher level than is optimal for basil growth due to requirements of other organisms in the system. Though no vegetative effects of the higher pH were observed in this 4-week experiment, chronic effects on plant growth are unknown. Initially, high levels of

ammonium (the water-soluble form of ammonia) and nitrites were present throughout the system, but both of these decreased over time to levels which were below the quantitation limit. This demonstrates that the fish were producing waste and bacteria were fixing that nitrogenous waste as expected. A curious point to note is how some of the ion concentrations of the hydroponic system seem to fluctuate over time, while those of the aquaponic system remain more stable. This is a result of the fact that hydroponics nutrients must be added multiple times a week, which lead to nutrient fluctuations, whereas in aquaponics the only input required is food for the fish with the remainder occurring naturally. The continuous addition of these nutrients may also explain why the hydroponic system has consistently higher ion concentrations than the aquaponic system. Additionally, the fact that certain ions (nitrate, phosphate, and sulfate) tend to accumulate in the hydroponic system may lead to chronic issues with plant viability. Some ionic species (nitrate, calcium, and potassium) also appear to be building up in the aquaponics system, but at a lower rate and at lower overall levels. Phosphate interestingly appears to slowly decrease, with some fluctuation over the study period in the aquaponics system. A longer-term study would more definitively show what the limit of these trends are if and when these ionic species reach a steady-state equilibrium.

Four of the twelve morphometric comparisons between basil plants in hydroponic and aquaponic systems differed. Of these four comparisons, two indicated a higher growth rate in hydroponic systems and two in aquaponic systems. These results neither support nor refute the hypothesis that basil would produce higher yields in an aquaponic system over a hydroponic system; indeed, the data instead suggest a more complicated tradeoff system that does not directly favor one system over another for leaf and stem growth. One needed area of future research is to examine morphometric comparisons after a full growing season, particularly after the aquaponic system has become more established. Other potential variables to compare might include vegetative fresh weight and dry matter production. Though there have been observations that aquaponic systems are not more productive than hydroponics until they have been established (~6 months), there have yet to be any empirical studies exploring this (Wilson 2005).

Interestingly, the two significant comparisons that indicated a higher yield in hydroponics were both measurements taken from young leaves at the 4-week mark—mean number of leaves and mean stem length. In contrast, the two significant comparisons that indicated a higher yield in aquaponics were both measurements taken from old leaves at the 2-week mark—leaf number and leaf density. This indicates that after two weeks of

growing, plants that were already slightly more established (old plants) were about the same height in both systems yet bushier in the aquaponic systems. In addition, after four weeks growing, plants that were less established to begin with (young plants) were taller with more leaves in hydroponics, but not any bushier than those in aquaponics. Future studies should examine the influence of both hydroponic and aquaponics systems on different-aged plants.

One factor that may have influenced the morphometric results was the amount of leaf tissue damage due to aphids. This unexpected variable influenced the aquaponic treatments more than the hydroponic treatments. A potential reason for this could be due to the substrate media of the grow beds—in hydroponics, grow beds were plastic square pipes with a soil plug in which the roots were anchored, whereas in aquaponics, plants were directly rooted in a porous rock grow media. Aphids were removed by hand from each system upon discovery. Though aphids falling from hydroponic plants landed on a flat surface and therefore could still be seen and removed, those falling from aquaponic plants landed into the rock bed and so could not be seen and further removed. Aphids from the aquaponic plants appeared to have a better chance at hiding and climbing back up the plant stalk than those on hydroponic plants. Whereas aphid damage was noted more in aquaponic basil plants, chlorosis was not detected. Chlorosis has been identified as a chronic problem in aquaponic basil production (Roosta 2014), but was not detected in either of our systems, possibly due to the limited growth time (4 weeks) of our experiment.

Results from the essential oil analysis suggest that the quality of basil is different in hydroponic and aquaponics systems, as leaves taken from plants in the hydroponic system had higher concentrations of the identified essential oils. One of these, eugenol, has potent antibacterial properties (Joshi 2014) and may be an indicator of stress in basil. Future work should examine the tentatively identified compounds with known standards to unambiguously identify the components as well as create calibration standards to accurately determine the concentrations of the major essential oil components. Whereas detailed analysis of individual plant variations in essential oils was beyond the scope of this project, future studies should quantify the different amounts of these compounds in aquaponic and hydroponic leaf samples. Indeed, the essential oil differences noted may have implications on basil flavor and health effects, and our findings warrant further examination.

In summary, this study, one of the first to compare basil production in aquaponic and hydroponic growing systems, noted several differences between the plant growth in each

system. Whereas the hypothesis that plants grown using aquaponics would have a higher yield was not supported, the stability of water chemistry and unique essential oil profile support the use of aquaponics to grow basil. These factors, along with the long-term stability of the koi-based aquaponics system, should be examined further.

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